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Biochimica et Biophysica Acta 1763 (2006) 1413–1426
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Review

Peroxisomal β-oxidation—A metabolic pathway with multiple functions

Yves Poirier [a], Vasily D. Antonenkov [b], Tuomo Glumoff [b], J. Kalervo Hiltunen [b,*]

[a] *Department of Plant Molecular Biology, Biophore, University of Lausanne, CH-1015 Lausanne, Switzerland*
[b] *Biocenter Oulu and Department of Biochemistry, University of Oulu, POB 3000, FIN-90014 Oulu, Finland*

Received 5 May 2006; received in revised form 21 August 2006; accepted 23 August 2006
Available online 30 August 2006

Abstract

Fatty acid degradation in most organisms occurs primarily via the β-oxidation cycle. In mammals, β-oxidation occurs in both mitochondria and peroxisomes, whereas plants and most fungi harbor the β-oxidation cycle only in the peroxisomes. Although several of the enzymes participating in this pathway in both organelles are similar, some distinct physiological roles have been uncovered. Recent advances in the structural elucidation of numerous mammalian and yeast enzymes involved in β-oxidation have shed light on the basis of the substrate specificity for several of them. Of particular interest is the structural organization and function of the type 1 and 2 multifunctional enzyme (MFE-1 and MFE-2), two enzymes evolutionarily distant yet catalyzing the same overall enzymatic reactions but via opposite stereochemistry. New data on the physiological roles of the various enzymes participating in β-oxidation have been gathered through the analysis of knockout mutants in plants, yeast and animals, as well as by the use of polyhydroxyalkanoate synthesis from β-oxidation intermediates as a tool to study carbon flux through the pathway. In plants, both forward and reverse genetics performed on the model plant *Arabidopsis thaliana* have revealed novel roles for β-oxidation in the germination process that is independent of the generation of carbohydrates for growth, as well as in embryo and flower development, and the generation of the phytohormone indole-3-acetic acid and the signal molecule jasmonic acid.

Keywords: β-oxidation; Metabolic compartmentalization; Fatty acid; Peroxisome; Multifunctional enzyme; Intermediary metabolism

1. Introduction

Living organisms are exposed to a large variety of fatty acids and their derivatives that can arise from either exogenous sources or endogenous synthesis. The fatty acids and their CoA esters play multiple roles in cellular processes by serving as components in cellular lipids, carbon storage as triacylglycerols or polyhydroxyalkanoates (in certain bacteria), regulators of enzymes and membrane channels, ligands for nuclear receptors, precursor molecules for hormones, signaling molecules including second messengers, and substrates for α-, β-, and ω-oxidations. Quantitatively, the major degradative pathway for fatty acid esters is β-oxidation, which in animal cells takes place in both mitochondria and peroxisomes and thus is an example of metabolic compartmentalization. All peroxisomes appear to accommodate fatty acid β-oxidation which, in most fungi as well as in plants, seems to be solely a peroxisomal process.

Both mitochondrial and peroxisomal β-oxidation catalyze the chain shortening of acyl-CoA esters between carbons 2 and 3, yielding as products chain-shortened acyl-CoA and acetyl-CoA or propionyl-CoA, depending on substrates. In both organelles, degradation of straight-chain saturated fatty acids requires the participation of the four activities comprising the core of the β-oxidation cycle, namely acyl-CoA dehydrogenase in mitochondria or acyl-CoA oxidase in peroxisomes, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase and the reactions proceed via 2E-enoyl-CoA, a chiral 3-hydroxyacyl-CoA and 3-ketoacyl-CoA intermediates. In contrast, degradation of fatty acids with double bonds typically require the participation of auxiliary enzymes, including Δ^3,Δ^2-enoyl-CoA isomerase, $\Delta^{3,5}$,$\Delta^{2,4}$-dienoyl-CoA isomerase and 2,4-dienoyl-CoA reductase [1,2]. In addition to straight-chain saturated and unsaturated fatty acids, the substrates of peroxisomal β-oxidation can be acyl moieties carrying

* Corresponding author. Fax: +385 8 553 1141.
E-mail address: kalervo.hiltunen@oulu.fi (J.K. Hiltunen).

0167-4889/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2006.08.034

hydroxyl or methyl groups, or bulky modifications like a steroid nucleus [3,4] as well as phenoxy, indole or cyclopentanone groups [5]. The substrate multiplicity sets requirements regarding the catalytic properties of the enzymes in the pathway. To meet these requirements, the peroxisomal β-oxidation pathway has adopted several strategies. These include evolution of enzymes towards a wide range of substrate specificities, evolution of β-oxidation enzymes as paralogues showing specificities with respect to their substrates, as well as recruiting and optimizing enzymes from non-homologous sources allowing them to catalyze a parallel set of reactions with different substrate specificities [2]. The adaptation has been fine-tuned by the development of systems controlling expression of genes encoding β-oxidation enzymes in response to exposure of the organism to different lipids, hormonal status, environmental stress or developmental stages.

A part of the activities operating in the β-oxidation pathway are catalyzed by multifunctional enzymes (MFEs) that carry out consecutive chemical modifications. At least in some cases, the metabolites are predicted to shuttle from one active centre to the next without being detached from the protein [6–9]. This substrate channelling provides efficient processing of metabolites and ensures that they undergo all modifications provided by the specific MFE.

The flux through β-oxidation requires reoxidation of NADH and transfer of metabolites across the peroxisome membrane. The permeability properties of the peroxisomal membrane, peroxisomal channels and transporters as well as peroxisomal disorders are discussed in depth elsewhere in this issue. Although these aspects are linked to the theme of this article, we will focus on the physiological function, current progress in structural enzymology and enzymes linking modified acyl-CoAs to peroxisomal β-oxidation.

2. Contribution of peroxisomal β-oxidation to the metabolism

Peroxisomal β-oxidation in fungi, such as *Saccharomyces cerevisiae*, is primarily devoted to the degradation of (often extracellular) fatty acids for the subsequent use of acetyl-CoA as a carbon and energy source for growth, although β-oxidation was also shown to be involved in the endogenous turnover of intermediates leaking out of the fatty acid biosynthetic pathway [10]. Although the role of β-oxidation as a principal provider of carbon and energy is also true for plants and animals, β-oxidation in these hosts also has other physiological roles.

Much of our understanding on the physiological roles of mammalian peroxisomal β-oxidation is based on the *in vitro* characterization of kinetic properties of peroxisomal enzymes, the identification of inborn deficiencies in peroxisomal fatty acid oxidation and analyses of metabolite profiles in affected individuals as well as generation of genetically modified organisms [11,12]. Patients with a defect in peroxisomal β-oxidation show neurological symptoms which are present at birth or develop during childhood, but occasionally the symptoms develop only after adolescence. A hallmark of peroxisomal β-oxidation in rodents, especially in liver tissue, is the high inducibility of a particular set of enzymes of the pathway, but not all, in a peroxisome proliferator receptor (PPAR) α-dependent manner by feeding the animals with peroxisome proliferators [13].

Long and medium chain-length unsaturated and saturated fatty acids are well accepted as substrates by mitochondrial and peroxisomal β-oxidations. There are, however, a set of fatty acids and their derivatives which in mammals are practically β-oxidized only by the peroxisomal pathway. These compounds include long-chain dicarboxylic and very long-chain monocarboxylic fatty acids. Others are certain leukotriens and prostaglandins, carboxylic derivatives of some xenobiotics, isoprenoid-derived fat soluble vitamins, and pristanic acid, a product of the α-oxidation of phytanic acid. These various compounds with long aliphatic carbon chain, which are often poorly soluble in water, are transformed to more polar metabolites in peroxisomal β-oxidation thus facilitating their elimination. Although the mitochondria have the same enzyme machinery to metabolize all the double bonds of polyunsaturated fatty acids, experiments with isolated organelles have demonstrated that contrary to mitochondrial β-oxidation, polyunsaturated fatty acids are well oxidized in peroxisomes and slowly oxidized in mitochondria [14]. Some of these acids can even inhibit the fatty acid β-oxidation in mitochondria [15]. Low levels of 4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid [22:6(n-3)] are found in tissues of Zellweger patients and mice with deficient peroxisomes. These findings were originally taken as a suggestion for the existence of a (peroxisomal) Δ4-desaturase. However, this desaturase has not been found in mammals, and according to the current view 22:6(n-3) can be generated via a retroconversion pathway that includes chain-shortening of 24:6(n-3) by one cycle through peroxisomal β-oxidation [4,16]. The contribution of mammalian peroxisomal β-oxidation in the generation of C24-bile acids from C27-hydroxysteroids derivatives of cholesterol is reviewed elsewhere in this issue.

Our understanding of the physiological role of β-oxidation in plants has been expanded considerably in the last few years. The identification of mutants, through forward and reverse genetics, in several β-oxidation enzymes has confirmed the expected role of these enzymes in the degradation of reserve lipids present in seeds of plants such as *Arabidopsis thaliana* (see next subheadings for references and further details). However, these same mutants have also revealed novel roles for β-oxidation, including a requirement for the emergence of the radicle from the seed coat, embryo and flower development, production of jasmonic acid involved in wounding response and the phytohormone indole-3-acetic acid.

Beyond its role in the breakdown of storage lipids, β-oxidation in plants has been shown to be active in a variety of tissues, including the developing seed of *A. thaliana* that rapidly synthesizes fatty acids and triacylglycerides [17]. The turnover of fatty acids via β-oxidation is particularly enhanced in plants synthesizing unusual fatty acids, such as capric acid, vernolic acid and ricinoleic acid, and underlies endogenous mechanisms involved in recycling and keeping such unusual fatty acids out of membrane lipids [18,19].

3. Acyl-CoA oxidases

In the peroxisomal β-oxidation cycle, the first reaction is catalyzed by an acyl-CoA oxidase (ACOX) which is regarded to be the main enzymatic step controlling the flux through the pathway. All the known peroxisomal ACOX enzymes are dimeric FAD-containing proteins which belong to the same superfamily as tetrameric mitochondrial acyl-CoA dehydrogenases. The typical feature of the superfamily is the central β-strand domain followed by an α-helical domain to which FAD is bound [20]. The electrons from FAD in ACOX enzymes are transferred directly to oxygen to generate H_2O_2 whereas the mitochondrial acyl-CoA dehydrogenases pass electrons to the respiratory chain via electron transferring flavoprotein.

The substrate diversity accepted in the peroxisomal β-oxidation pathway is exemplified by the existence of three acyl-CoA oxidase genes in mammals. The rodent ACOX1 (palmitoyl-CoA oxidase) is inducible in livers by peroxisome proliferators and catalyzes the oxidation of long and medium straight chain substrates but shows very low activities towards short chain substrates [21]. This kinetic property of mammalian ACOX1 agrees well with the data obtained by many laboratories with isolated peroxisomal enzymes *in vitro*, namely the β-oxidation of straight chain fatty acids in mammalian peroxisomes does not proceed to completion. This notion is supported further by the presence of carnitine octanoyl transferase in peroxisomes [22,23] providing a carnitine dependent mechanism for export of medium chain fatty acid generated in mammalian peroxisomes. Disruption of *Acox1* in mice results in infertility, retarded postnatal growth and liver abnormalities [24]. The other two oxidases in rat liver are named pristanoyl-CoA oxidase (ACOX2) and cholestanoyl-CoA oxidase (ACOX3), based on their substrate specificities. In humans, di- and trihydroxycholestanoyl-CoAs are oxidized by the same oxidase that oxidizes the other branched-chain acyl-CoA esters. Humans also have a third acyl-CoA oxidase gene that is a homolog of the rat ACOX3, but this gene appears to be non-functional [25].

S. cerevisiae has only one peroxisomal acyl-CoA oxidase, Pox1p/Fox1p, and therefore *pox1*Δ mutant cells are unable to grow on oleic acid as the only carbon source. The complete β-oxidation of oleic acid suggests that the active site of Pox1p/Fox1p has acquired a broad ligand binding property concerning the chain length of the acyl group in substrates. Unlike *S. cerevisiae*, many other yeasts like *Candida tropicalis* or *Yarrowia lipolytica* have several acyl-CoA oxidases with different chain length specificities [26].

Although acyl-CoA oxidases have been identified and cloned from a few plant species, the best description has been obtained from *A. thaliana*. The genome of *A. thaliana* contains 6 genes showing high homology to the mammalian acyl-CoA oxidases, of which only 4 have been characterized biochemically. ACX1 is a medium- to long-chain acyl-CoA oxidase with a substrate optimum of C14:0, ACX2 has optimum activity with long-chain saturated and unsaturated acyl-CoAs (C14:0 to C20:0), ACX3 exhibits medium-chain substrate specificity (C8:0–C14:0) and ACX4 exhibits short-chain substrate specificity (C4:0–C8:0) [27–30]. The presence of a short-chain acyl-CoA oxidase enables to complete k-oxidation of fatty acids in plant peroxisomes, similar to fungal peroxisomes. Interestingly, phylogenetic analysis showed that ACX4 clusters with mitochondrial acyl-CoA dehydrogenase rather than acyl-CoA oxidase, suggesting that it arose from an acyl-CoA dehydrogenase that acquired a peroxisomal targeting sequence during evolution [27]. The X-ray structure of ACX1 revealed that the enzyme is a dimer and that it has a fold resembling that of mammalian acyl-CoA oxidase [31].

Lipid degradation during germination and subsequent post-germinative growth was found to be unaffected in an *A. thaliana acx1* knockout mutant and only slightly delayed in an *acx2* mutant, indicating the partially redundant nature of these enzymes [32]. However, the double mutant *acx1/acx2* was found to be unable to catabolize storage lipids and accumulated long-chain acyl-CoAs, in line with an early block of the β-oxidation cycle. Post-germinative seedling growth and establishment of photosynthetic competency were severely compromised in the double mutant, but were normal if sucrose was provided to the media, indicating that the effect of the blockage of the β-oxidation on post-germinative growth was mainly related to the provision of carbon and energy for growth. Interestingly, it was also found that the double mutant had a significant decrease in germination frequency that could not be alleviated by addition of exogenous sucrose, demonstrating a role for β-oxidation in germination that goes beyond the simple provision of carbon and energy [32].

While no phenotypes have been observed throughout the life cycle in the single mutants *acx3* and *acx4*, despite the large reduction in medium- and short chain acyl-CoA oxidase activity, respectively, the double mutant *acx3/acx4* aborted during the first phase of embryo development, suggesting an important role for the β-oxidation of short-chain acyl-CoA esters in development [33].

Indole-3-butyric acid (IBA) is an endogenous plant hormone of the auxin class that can be converted to indole-3-acetic acid (IAA) via one round of β-oxidation. Several mutants shown to be resistant to the effects of IBA on root growth were deficient in enzymes of the β-oxidation cycle [34]. Similarly, 2,4-dichloro-phenoxy-butyric acid (2,4-DB) can be converted by β-oxidation to 2,4-dichloro-phenoxy-acetic acid (2,4-D), which has herbicide and auxin-like activity in plant cells, and plants resistant to the action of 2,4-DB were blocked in various steps of β-oxidation. The mutants *acx1*, *acx3* and *acx4* have increased resistance to IBA, while only *acx3* and *acx4* were more resistant to 2,4-DB [32,35]. Although the link between resistance to IBA/2,4-DB action and the β-oxidation has been shown repeatedly, the fact that mutants in acyl-CoA oxidases having different chain-length specificities, such as *acx1* and *acx4*, show similar resistance to IBA suggests that the response defects to IBA, and perhaps also to 2,4-DB, in these mutants may be a more indirect block on peroxisomal metabolism than a direct implication of acyl-CoA activity on these substrates [35].

Jasmonic acid (JA) is an important signalling molecule involved in the response of plants to wounding and pathogen attack. JA synthesis implicates the β-oxidation of the precursor

molecule 3-oxo-2-(2′-pentenyl)-cyclopentane-1-octanoic acid (OPC:8). Of all four *A. thaliana acx* mutants tested, only *acx1* showed a decrease in JA accumulation after wounding, implicating more directly the action of ACX1 in synthesis of JA [32,36]. Mutants in which the homologous tomato protein LeAPX1A was deficient, also revealed lack of JA synthesis in leaves, and recombinant LeAPX1A was shown to be active on OPC:8 [37].

4. Multifunctional enzymes

Similarly to the mammalian inner membrane bound mitochondrial or bacterial cytoplasmic β-oxidations, the peroxisomal system includes MFE which catalyzes the second and the third reactions of the pathway (Fig. 1A). The simultaneous existence of MFE-1 (also known as L-bifunctional protein, LBP) and MFE-2 (D-bifunctional protein, DBP) in mammalian peroxisomes catalyzing the same set of reactions via mirror image stereochemistry is intriguing [38]. They enjoy a substantial scientific interest because they are not sequence related, have different evolutionary origins and thus are representatives of functional convergence. Their enzymatic and functional properties are different, their interdomain organization shows unique features as reflected by the substrate profiling and predicted channeling between catalytic sites in different domains.

4.1. Peroxisomal multifunctional enzyme type 2 (MFE-2)

MFE-2 proteins from yeasts [39] and mammals [40] harbor in each case three functional domains in one polypeptide

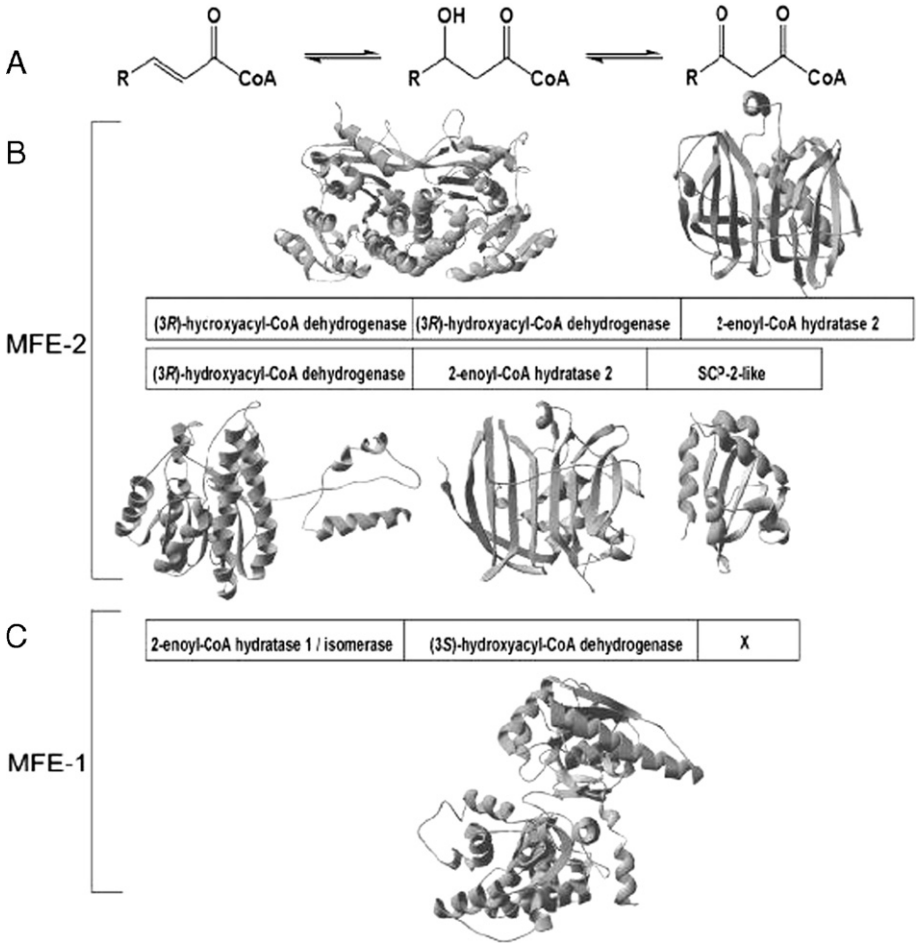

Fig. 1. Structural information of eukaryotic peroxisomal multifunctional enzymes type 1 (MFE-1) and type 2 (MFE-2). (A) Basic reactions catalyzed by the MFEs. 2E-enoyl-CoAs (left) serve as substrates for the 2-enoyl-CoA hydratases producing 3-hydroxyacyl-CoAs (middle), which in turn are transformed to 3-ketoacyl-CoAs (right) by the 3-hydroxyacyl-CoA dehydrogenases. MFE-1 and MFE-2 perform opposite stereochemistry with respect to the 3-hydroxy group with MFE-1 being specific for the S-stereoisomer and MFE-2 for the R-isomer. (B) Composition of MFE-2 polypeptides from their functional domains is shown as schematic rectangles in the middle with the N-terminus on the left and the C-terminus on the right. The upper rectangle indicates the presence of two 3-hydroxyacyl-CoA dehydrogenases and one 2-enoyl-CoA hydratase in a typical yeast (*Candida tropicalis*) MFE-2, while the lower rectangle shows the corresponding structure for human MFE-2 with one of each enzymatic domain followed by a domain homologous to sterol carrier protein 2. The corresponding domain crystal structures are shown above and below the rectangles as ribbon drawings with one monomer per structure indicated. The structures have been determined by producing the domains as separate proteins. (C) Composition of human MFE-1 polypeptide from its functional domains with a combined 2-enoyl-CoA hydratase 1/isomerase in the N-terminus, a 3-hydroxyacyl-CoA dehydrogenase in the middle and a domain "X" with unknown function in the C-terminus. The crystal structure of the dehydrogenase domain is known and is shown as a ribbon drawing below the polypeptide structure. The Protein Data Bank (www.rcsb.org) accession codes for the atomic coordinates of the determined crystal structures used in the drawings are (from left to right and from up to down) 2et6, 1pn4, 1zbq, 1s9c, 1ikt and 1zcj, respectively.

chain, of which either two or three have enzymatic activities. The function of MFE-2 is crucial not only for fatty acids utilization via β-oxidation, but also for lipid metabolism in general [11], as this enzyme contributes to the synthesis of bile acids and docosahexaenoic acid [12,16]. Recently, a genotype-phenotype correlation for D-bifunctional protein deficiency was demonstrated by structural analysis of MFE-2 domain crystal structures in the light of mutational data from a patient cohort [41]. MFE-2 protein in patients with milder symptoms was found to contain point mutations in locations that disturb the structural and functional architecture of the protein less than such mutations that are known to cause total inactivity of MFE-2, and thus severe disease symptoms.

The structure of the dehydrogenase domain of MFE-2 is known from rat [42], human [43] and the yeast *C. tropicalis* [44]. The overall structures are all identical with a notable 60-residue C-terminal extension to the Rossmann-fold core not found in other structurally related proteins. Differences in structural details are seen corresponding to subtle functional differences, mainly concerning substrate specificities. At the tertiary/quaternary structural levels, an immediate observation from the dimeric dehydrogenases in a dimeric MFE-2 protein is that the C-terminal domain from one monomer folds on the top of the substrate binding tunnel of the other monomer, and *vice versa*. This way the monomers contribute to the active site architectures of each other in a cooperative manner, strengthening also the monomer–monomer interactions. This structural feature gains even more importance in the case of yeast dehydrogenase dimers, where the two dehydrogenase units are part of the *same* polypeptide chain due to a duplication (Fig. 1B). There the necessity for cooperation in substrate binding is especially clearly demonstrated and correlated with the structure [44,45], namely the dehydrogenase A cannot use short chain fatty-acyl-CoAs as substrates, while the dehydrogenase B accepts these more readily than the long chain ones.

The structures of the yeast *C. tropicalis* [46] and human [47] hydratase 2 domains of MFE-2 were the first eukaryotic (*R*)-specific hydratase structures determined (Fig. 1B). Comparison with a bacterial (*R*)-hydratase [48] revealed the structural basis for the adaptation of the eukaryotic enzyme to accept long chain substrates: the absence of a long central α-helix from one of the two "hot-dog" folds (the N-terminal one) leaves half of the dimeric protein without enzymatic activity, but creates an extra binding space for bulky substrates, contrary to the bacterial enzyme that is half the size, it forms homodimers and binds a short chain substrate with every such fold. Thus, the adaptation of the eukaryotic protein for bulky substrates has happened in the evolution with a cost of one active site per equal volume of polypeptide chain.

A substantial controversy surrounds the sterol carrier protein 2-like domain of MFE-2. Various gene fission and fusion events have resulted in MFE-2 proteins that either combine SCP-2 differently or have it as a separate protein: for example, *C. elegans* MFE-2 combines it with the dehydrogenase domain leaving the hydratase 2 a separate protein, while *D. melanogaster* combines both enzymatic domains leaving SCP-2 aside [49]. This domain is from the structural point of view nothing much but a hydrophobic binding pocket (Fig. 1B) capable of binding even such clearly non-physiological molecules as Triton X-100 [50] in addition to sterol and fatty acid/acyl-CoA-type ligands relevant for β-oxidation [51]. The role of a domain homologous to SCP-2 as part of MFE-2 is not known.

Despite the lack of structural data from full-length MFE-2 proteins, plausible models based on the domain crystal structures have been presented [44,47]. These models are consistent with the biochemical data, and include the idea that the domain contacts in mammalian and fungal MFE-2s must be different (due to the different array of functional units), and a possibility for a selective substrate channeling mechanism in yeast MFE-2.

Search of the *A. thaliana* genome for proteins showing homology to the yeast and mammalian MFE-2 identified ECH2, a protein of 34 kDa showing 40% and 34 amino acid % identity to the human and fungal enoyl-CoA hydratase 2 domain of MFE-2, respectively (S. Goepfert and Y. Poirier, unpublished). The protein contains no domain homologous to the 3R-hydroxyacyl-CoA dehydrogenase domain of yeast or mammalian MFE-2. The protein is targeted to the peroxisomes in both yeast and plants. Enzymatic assays confirmed that ECH2 is a monofunctional enoyl-CoA hydratase-2 catalyzing the reversible conversion of 2E-enoyl-CoA to 3R-hydroxyacyl-CoA (S. Goepfert, K. Hiltunen and Y. Poirier, unpublished). ECH2 was shown to participate *in vivo* in the metabolism of fatty acids with a *cis* unsaturated bond on an even-numbered carbon by the conversion of the intermediate 3R-hydroxyacyl-CoA, generated via the hydration of 2Z-enoyl-CoA by the MFE-1, to the 2E-enoyl-CoA for further degradation through the core β-oxidation cycle.

4.2. Peroxisomal multifunctional enzyme type 1 (MFE-1)

Peroxisomal MFE-1 was first identified from rat [52,53]. It is one of the most abundant proteins in rodent peroxisomes and has been a target in a large number of studies. In spite of 25 years of efforts, its physiological function remained concealed and just during last years some light has been shed on its role in metabolism.

MFE-1 is a monomeric 79 kDa protein showing 2-enoyl-CoA hydratase 1, 3S-hydroxyacyl-CoA dehydrogenase as well as Δ^3,Δ^2-enoyl-CoA isomerase activities. Amino acid sequence comparisons with the monofunctional 2-enoyl-CoA hydratase 1 (crotonase), Δ^3,Δ^2-enoyl-CoA isomerase and 3S-hydroxyacyl-CoA dehydrogenase together with the studies exploiting engineered MFE-1 variants [54] have located hydratase 1/isomerase activities to the N-terminal and dehydrogenase to C-terminal halves, respectively (Fig. 1C). Thus its homologues in mitochondria are the α-chain of the membrane-associated trifunctional enzyme and in bacteria the α-chain of the soluble $\alpha_2\beta_2$ fatty acid β-oxidation complex (FOM). Based on multiple sequence alignment and mutational analysis, the MFE-1 can be divided into five distinct domains referred as to A, B, C, D, and E. Domain A provides the core of the hydratase/isomerase fold and it has a shared active site pocket for hydration and isomerisation. The crystal structure revealed that

the B domain of MFE-1 corresponds to the αFOM linker helix of *Pseudomonas fragi* FOM (PtFOM) [9], located between its hydratase and dehydrogenase part.

The recent 1.9 Å crystal structure of the dehydrogenase part of the rat MFE-1 [55] revealed that the domain C is the dinucleotide binding domain present in the short-chain acyl-CoA dehydrogenase family (SCHAD). Domain D is similar to the dimerization domain of SCHAD. Domain E is about 120 amino acid residues long, carries the type 1 peroxisomal targeting signal (PTS1) SKL, and has low sequence similarity with dimerization domain of SCHAD suggesting that it arose via a partial gene duplication. In MFE-1 a local 2-fold axis, corresponding to the SCHAD dimer 2-fold axis, relates domain D to the domain E and therefore this domain E prevents the MFE-1 monomer from dimerizing similarly to in the SCHAD dimer.

Similarly to MFE-2, MFE-1 shows broad substrate specificity. It accepts short and long chain enoyl-CoA esters, CoA esters of hydroxylated C27 bile acid synthesis intermediates and dicarboxylic acids. No patients with MFE-1 deficiency has been described, and *Mfe-1* null mice do not show appreciable changes in lipid metabolism and they are symptomless [56]. The inactivation of the *Amacr* gene, encoding α-methylacyl-CoA racemase (Amacr) in mice will disrupt the generation of bile acids via Amacr-MFE-2-dependent pathway. The observed residual C24 bile acids, about 20% of that found in the wild type mice, indicated the existence of a parallel pathway. The kinetic properties of MFE-1 allows together with CYP3A11 and CYP46A1 to make an Amacr-independent pathway for the generation of C24 bile acids [57].

It has been demonstrated that the rat peroxisomal MFE-1 can rescue the growth of a *mfe-2* null mutant yeast strain on oleic acid which results in the change of stereochemistry of the yeast endogenous β-oxidation pathway [38]. However, in humans this complementation can be at most partial, because patients with a deficiency in MFE-2 fail to thrive. A new aspect on the physiological role of MFE-1 was recently shown by the demonstration that the primary function of MFE-1 may be involvement in oxidation of dicarboxylic acids [58].

MFE activity has been studied in cucumber, where four different isozymes were characterized [59,60]. The gene encoding for the 76.5 kDa isozyme was cloned and the protein was shown to contain, in addition to a 2E-enoyl-CoA hydratase and a 3S-hydroxyacyl-CoA dehydrogenase, a Δ^3,Δ^2-enoyl-CoA isomerase and a 3R-hydroxyacyl-CoA epimerase activity [61]. The epimerization of 3R-hydroxyacyl-CoA was shown to be mediated by an epimerase rather than the combined activity of a R- and S-specific hydratase by the identification of an N-terminal fragment lacking crotonase activity but retaining Δ^3,Δ^2-enoyl-CoA isomerase and a 3R-hydroxyacyl-CoA epimerase activity [61]. The *A. thaliana* genome contains only 2 genes, named *AIM1* and *MFP2*, which encode proteins having extensive homology to the cucumber MFE-1. While AIM1 is mostly expressed in siliques and flowers and shows little expression during germination and early post-germinative growth, MFP2 shows significant induction during germination and is highly expressed during early post-germinative growth [62,63]. The mutant *aim1* showed normal germination, seedling establishment and growth of the leaves and rosettes [63]. However, upon transition to reproductive growth, the *aim1* mutant showed abnormal flower development resulting in severely reduced fertility. The *aim1* mutant also had increased resistance to the herbicide 2,4-DB. In contrasts, the mutant *mfp2* showed a sucrose-dependent reduction in post-germinative seedling establishment that could be rescued by sucrose addition in the media, but was not different from wild type for 2,4-DB resistance [64]. The *mfp2* seedlings catabolized storage lipids more slowly, accumulated long-chain acyl-CoAs and the peroxisomes were increased in size. However, when photosynthesis was established, the *mfp2* plant grew like wild type and produced flowers and seeds normally. Interestingly, the double mutant *aim1/mfp2* aborted during early stages of embryo development, in a manner similar to the double mutant *acx3/acx4*. Together, these results demonstrate that the physiological role of AIM1 and MFP2 can be quite distinct although some overlaps exist during embryo development. It is unknown at this point how the lack of AMI1 leads to abnormal flower development or how the lack of both AIM1 and MFP2 blocks embryo development. It is possible that β-oxidation may either decrease the amount of a compound inhibiting flower or embryo development or be involved in the generation of a molecule required for progression through these developmental stages.

5. 3-Ketoacyl-CoA thiolases

The last reaction of the β-oxidation cycle is the thiolytic cleavage of 3-ketoacyl-CoA to acetyl-CoA and acyl-CoA shortened by two carbon atoms. The only X-ray structure available for peroxisomal β-oxidation thiolases is the yeast peroxisomal thiolase, Pot1p/Fox3p. This enzyme is a dimeric protein with a subunit size of 45 kDa, similar to the peroxisomal thiolases in other species, whereas the mitochondrial thiolases are tetrameric. The Pot1p/Fox3p subunit consists of three domains: two core domains, which have similar folds and a loop domain of about 120 residues. The core domain fold into a mixed five-stranded β-sheet, which is covered on each side by α-helices. The active site in yeast thiolase is surrounded by residues from two core domains and residues from the loop domain [65].

Rat peroxisomes have three thiolases. Thiolase A, which is constitutively expressed is active with straight chain substrates of varying acyl chain length. Thiolase B, which is inducible by peroxisome proliferators, has very similar properties as thiolase A with respect to the substrate acceptance profile. The third peroxisomal thiolase is SCP-2/3-ketoacyl-CoA thiolase (SCPx), which is a 58 kDa protein housing N-terminally the thiolase and C-terminally the SCP-2 domain [66–69]. After the import into peroxisomes, SCPx can be cleaved yielding 46 kDa thiolase and 13 kDa SCP-2 domains, respectively. SCP-2 can arise also separately because it has its own promoter on the *SCPx* gene. SCPx has very broad substrate specificity, including cleavage of 2-methyl branched as well as straight chain 3-ketoacyl-CoA esters. Humans have two thiolases: one is the human equivalent of the rat peroxisomal thiolase and the other one is the human

ortholog of SCPx. The enzyme activity studies *in vitro*, metabolite profile analysis of knockout mice and a recently described patient with SCPx deficiency [41] indicate that SCPx participates in the thiolytic cleavage of 2-methyl branched substrates leading to the degradation of pristanic acid and synthesis of bile acids.

Genes encoding 3-ketoacyl-CoA thiolases have been cloned from numerous plant species. The *A. thaliana* genome contains 3 genes encoding peroxisomal 3-ketoacyl-CoA thiolase, of which only one, namely KAT2/PED1, has been characterized in some detail [70]. KAT2 was shown to be the main 3-ketoacyl-CoA thiolase active during seed germination. Similar to the *acx1/acx2* double mutant, the *kat2* mutant was shown to have a sucrose-independent reduction in seed germination, while the strong delay in post-germinative seedling growth was found to be rescued by external sucrose, thus again indicating a distinct role for β-oxidation in germination and seedling growth [32,70]. The *ped-1* mutant was also more resistant to the action of IBA and 2,4-DB, and showed a decrease in the synthesis of jasmonic acid following wounding [34,36,70,71,72]. The substrate specificity of KAT2, as well as of the two other *A. thaliana* 3-ketothiolases KAT1 and KAT5, has not been studied. However, the activity of KAT2 towards acetoacetyl-CoA, the accumulation of long-chain acyl-CoAs in the *kat2* mutant as well as the implication of this protein in jasmonic acid synthesis suggest that KAT2 may have activity towards a broad range of substrates [36,70,72].

6. Auxiliary enzyme systems

The generation of chain-shortened products by peroxisomal β-oxidation can proceed only in the presence of mechanisms continuously removing them. According to the current view, the products leave peroxisomes either after hydrolysis to free acids by acyl-CoA thioesterases or transesterification (Fig. 2). The classic β-oxidation pathway is able to complete the breakdown of saturated fatty acids or those having *trans* double bonds at even-numbered position as well as 2S-methyl-branched fatty acids if a methyl-branched acyl-CoA oxidase is present. However, concerning *cis* or *trans* double bonds at odd-numbered positions, or *cis* double bonds at even-numbered positions, the degradation can be completed by combining the actions of k^3,Δ^2-enoyl-CoA isomerase, $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase and 2,4-dienoyl-CoA reductase together with the core enzymes of the β-oxidation pathway [1,73,74].

6.1. Thioesterases

Acyl-CoA thioesterases/hydrolases (ACOTs) are a group of enzymes that catalyze the hydrolysis of acyl-CoAs to the free fatty acid and CoA, providing the potential to regulate intracellular levels of acyl-CoAs, free fatty acids and CoASH. The ACOTS have at least 12 family members in mammals (ACOT1-ACOT12), which are localized in almost all cellular compartments such as endoplasmic reticulum, cytosol, mitochondria and peroxisomes [75]. Although the physiological functions of these enzymes are not yet fully understood, recent cloning and more in-depth characterization of acyl-CoA thioesterases has assisted in the discussion of functions for specific enzymes such as in the regulation and termination of fatty acid oxidation in mitochondria and peroxisomes. ACOT3 and ACOT5 have been characterized as peroxisomal acyl-CoA thioesterases showing preference toward long-chain and medium-chain acyl-CoAs, respectively [76]. ACOT8 shows a broad substrate specificity hydrolyzing straight and methyl-branched chain acyl-CoAs, dicarboxyl-CoAs as well as bile acid-CoA esters. This activity raises a question whether newly formed primary bile acids leave peroxisomes as free acids and subsequently undergo reactivation and conjugation extraperoxisomally. Recently it has been shown that ACOT4 is a peroxisomal highly specific succinyl-CoA thioesterase, suggesting that peroxisomal β-oxidation of dicarboxylic acids can also lead, in addition to medium chain-length dicarboxylic acids, to the formation of succinate [77].

S. cerevisiae contains a single peroxisomal acyl-CoA thioesterase, named Pte1p [78], which corresponds to ACOT8 in mice. The purified enzyme was shown to accept a broad range of substrates, ranging from acetyl-CoA to oleic acid and methyl-branched fatty acids [79]. While one study showed that deletion of the *PTE1* gene leads to a small but significant decrease in the ability of cells to grow on oleic acid as the main carbon source, no obvious effects were observed in a separate study with a similar mutant [78,80]. Recently, analysis of the carbon flux through the β-oxidation cycle *in vivo* using the synthesis of peroxisomal polyhydroxyalkanoate (PHA) from the polymerization of the 3R-hydroxyacyl-CoAs as a marker revealed that degradation of short straight-chain (heptanoic acid) and branched-chain fatty acids (8-methyl-nonanoic acid) was severely compromised in the *pte1*Δ mutant [79]. The poor catabolism of 8-methyl-nonanoic acid in *pte1*Δ cells had a negative effect on the degradation of normally well-metabolized fatty acids, such as 10Z-heptadecenoic acid, and reduced the ability of the cells to grow efficiently in media containing such fatty acids. These experiments provided direct evidence that the peroxisomal acyl-CoA thioesterase can be a key enzyme required for normal carbon flux through the core β-oxidation cycle, most likely by keeping a proper balance between free and CoA-esterified fatty acids, and maintaining an available pool of free CoA by preventing its sequestration into poorly metabolizable CoA esters.

6.2. 2,4-Dienoyl-CoA reductase

2,4-Dienoyl-CoA reductase has been purified from several sources including mammalian mitochondria and peroxisomes, yeast peroxisomes and bacteria [1,81]. The characterized reductases are NADPH-dependent enzymes, converting 2,4-dienoyl-CoA into 3E-enoyl-CoA end-product and, with exceptions the bacterial enzyme, are members of the SDR superfamily. The bacterial reductase is a flavin-containing enzyme that produces 2E-enoyl-CoA [82]. Clinical cases have been published, which clearly demonstrates the importance of 2,4-dienoyl-CoA reductase [83,84]. These patients show a decrease in reductase activity, but it remains unclear which isoform is defective. However, the ultimately fatal deficiency of one

isoenzyme shows that reductase activities cannot be replaced by other isoenzymes and that each of the isoforms has a unique metabolic role.

The yeast *S. cerevisiae* has only peroxisomal 2,4-dienoyl-CoA reductase encoded by the *SPS19* gene [85]. According to the suggested mechanism, reducing equivalents required in this NADPH-dependent reaction are transferred from the cytosol to peroxisomes by the α-ketoglutarate/isocitrate shuttle involving both cytosolic and peroxisomal NADP(H)-dependent isocitrate dehydrogenase [86].The *in vivo* requirement of the 2,4-dienoyl-CoA reductase for the degradation of fatty acids with a *cis* double bond on an even carbon has been demonstrated by the failure of the *sps19*Δ strain to grow on petroselenic acid as the main carbon source or to synthesize PHA on 10Z-heptadecenoic acid [85,87]. The *sps19*Δ mutant was shown to be competent to degrade the fatty acid 9E,12E-octadecadienoic acid when grown the cells on plates containing this fatty acid as a single carbon and energy source, indicating that the 2E ,4E-dienoyl-CoA intermediate is a suitable substrate for the MFE-2 and that the core β-oxidation is sufficient to degrade unsaturated fatty acids with a *trans* double bond on an even carbon [88]. However, analysis of the degradation of 10E-heptadecenoic acid using the synthesis of PHA as a tool to monitor β-oxidation revealed that in wild type cells, approximately half of the 10E-heptadecenoic acid was degraded via a pathway involving the 2,4-dienoyl-CoA reductase while the other half was degraded in a reductase-independent manner via the core β-oxidation [87]. Furthermore, while *sps19* mutant could degrade 10E-heptadecenoic acid, a significant reduction in the overall carbon flux through β-oxidation was observed in the *sps19*Δ mutant compared to wild type, revealing the requirement of the 2,4-dienoyl-CoA reductase for optimal degradation of such fatty acids.

A gene encoding a peroxisomal 2,4-dienoyl-CoA reductase from *A. thaliana* has recently been identified and shown to functionally complement the yeast *sps19*Δ (S. Goepfert and Y. Poirier, unpublished).

6.3. Δ^3,Δ^2-Enoyl-CoA isomerases

The Δ^3,Δ^2-enoyl-CoA isomerases are enzymes participating in the β-oxidation of unsaturated fatty acids in both

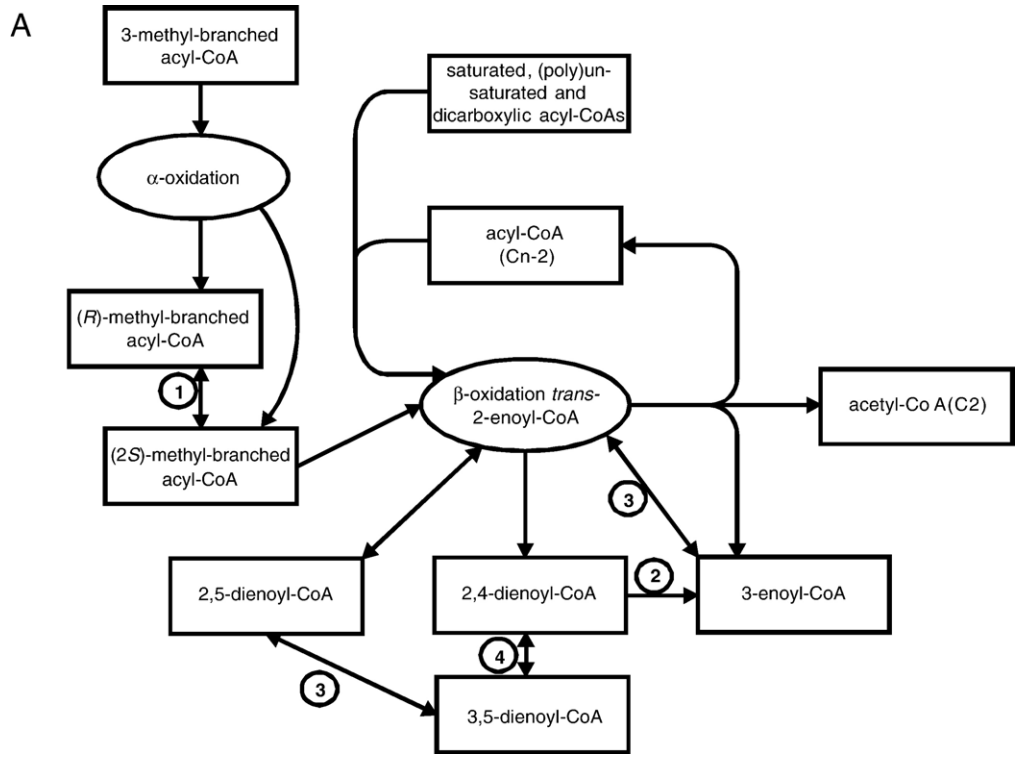

Fig. 2. Peroxisomal β-oxidation of fatty acyl-CoA, contributions of auxiliary enzyme systems to this process and fates of acetyl-CoA and chain-shortened acyl-groups generated for exit out of peroxisomes. (A) Methyl-branched fatty acids will be metabolized by peroxisomal á-oxidation and Amacr (1) to generate 2S-methylacyl-CoAs that serve as substrates for peroxisomal oxidases. When either 2,4-dienoyl-CoA, 2,5-dienoyl-CoA or 3-enoyl-CoA intermediates appear while β-oxidizing (poly)unsaturated fatty enoyl-CoAs, they enter into pathways catalyzed by 2,4-dienoyl-CoA reductase (2), Δ^3,Δ^2-enoyl-CoA isomerase (3), and $\Delta^{3,5}$, $\Delta^{2,4}$-dienoyl-CoA isomerase (4). In yeast, 2,5-dienoyl-CoAs can also be metabolized directly by the MFE-2 of the core β-oxidation cycle. Acyl-CoA thioesterases are likely to act on several acyl-CoAs and β-oxidation intermediates. (B) Dependent on species and organs, the generated acetyl-CoA can be condensed to oxaloacetate to form citrate by citrate synthase, condensed to glyoxylate to form malate by malate synthase, transferred to carnitine by carnitine acetyltransferase, or hydrolyzed to form free acetate by acetyl-CoA thioesterase. The generated medium-chain fatty acyl group can be transferred to carnitine by the carnitine octanoyltransferase or hydrolyzed to free acids by acyl-CoA thioesterase. Succinyl-CoA, arising as a product of degradation of dicarboxylic acids, can be converted to succinate by a succinyl-CoA thioesterase. The generated C24 bile acyl-CoA can leave peroxisomes after being hydrolyzed to free acids by an acyl-CoA thioesterase. The data on whether there exists also peroxisomal bile acid conjugating enzyme (BAAT) in addition to the cytoplasmic activity are contradictory. The CoA esters of jasmonic acid and indole-3-acetic acid can presumably be converted into the corresponding free acids by an acyl-CoA thioesterase in plants.

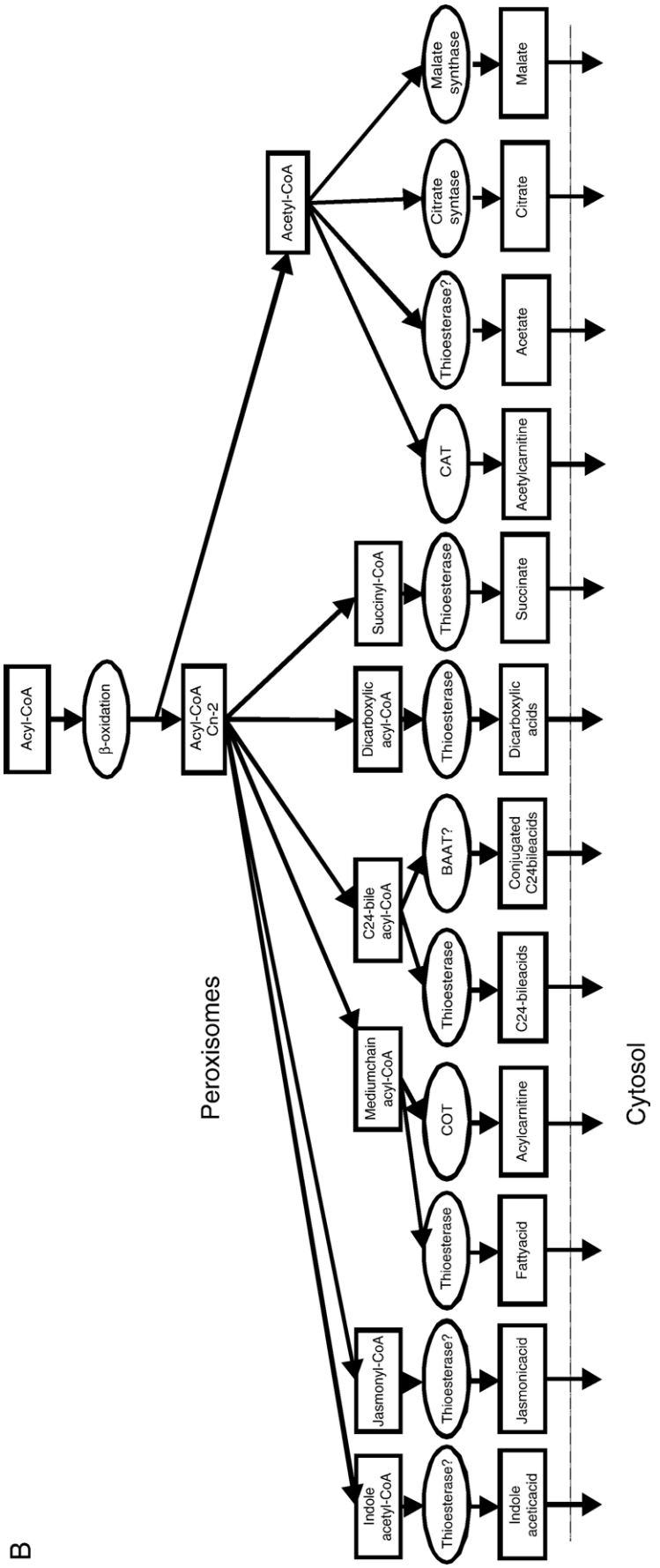

Fig. 2 (*continued*).

mitochondria and peroxisomes [89,90]. Each of these enzymes can catalyze the isomerisation of the 3Z and the 3E substrate molecules of various acyl chain lengths into 2E-enoyl-CoA. Mammalian cells have three isomerases each of them being a member of isomerase/hydratase 1 superfamily: monofunctional mitochondrial and peroxisomal enzymes and an isomerase as a part of peroxisomal MFE-1 sharing the same catalytic site as hydratase 1 [7,91]. Mammalian peroxisomal monofunctional isomerase (PECI) of human and mice has in addition to the hydratase/isomerase-like sequence, an extra domain about 80 amino acids in length at its N-terminus showing sequence similarity to the acyl-CoA binding protein (ACBP). ACBP is predicted to be involved in the intracellular acyl-CoA transport [20].

Two isomerase structures have been solved so far: the yeast peroxisomal enzyme, which is a hexamer, consisting of a dimer of trimers [92,93] and human and rat mitochondrial enzyme, which is a trimer [94,95]. The percentage sequence identity between the yeast peroxisomal isomerase and the human mitochondrial isomerases (13%) is very low. However, the CoA moiety is always bound in the same way, such that the thioester oxygen binds in a conserved oxyanion hole formed by two peptide NH-groups.

The most extensive enzymological characterization has been done by Zhang et al. [96] who have reported on the kinetic constants of rat mitochondrial and peroxisomal isomerases. The mammalian peroxisomal MFE-1 catalyzes, among the other isomerase reactions, the conversion of 2E,5Z-dienoyl-CoA into 3E,5Z-dienoyl-CoA. The product is a substrate for dienoyl-CoA isomerase and thus the participation in dienoyl-CoA isomerase-dependent degradation of (poly)unsaturated fatty acids has been proposed *in vivo* function of peroxisomal MFE-1 in mammals. Noteworthy, when the rat MFE-1 was expressed heterologously in ECI1-deleted yeast strain, the growth on oleic acid was restored, demonstrating that Δ^3,Δ^2-enoyl-CoA isomerisation is functional *in vivo* [97].

Three genes encoding monofunctional peroxisomal Δ^3,Δ^2-enoyl-CoA isomerases have been identified in *A. thaliana* and shown to complement the yeast *eci1*Δ mutant using the PHA assay with cells grown on 10Z-heptadecenoic acid (S. Goepfert and Y. Poirier, unpublished).

6.4. $\Delta^{3,5},\Delta^{2,4}$-Dienoyl-CoA isomerase

It was originally shown by Tserng and coworkers [98] that β-oxidation of unsaturated fatty acids with double bond at odd-numbered positions can be metabolized via an NADPH-dependent pathway in rat liver involving $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase [99]. Mammals have apparently only one gene encoding this protein which can be targeted by N- or C-terminal signal sequences into either mitochondria or peroxisomes [100]. The enzyme belongs to the isomerase/hydratase superfamily and its crystal structure has been solved at the 1.5 Å resolution [101].

The *S. cerevisiae* genome contains a single gene, *Dci1*, encoding a peroxisomal $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase. Dci1p was shown to have a weak intrinsic Δ^3,Δ^2-dienoyl-CoA isomerase activity in addition to a $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase activity [102]. The *in vivo* contribution of the $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase to the degradation of unsaturated fatty acids remains enigmatic, since *dci1*Δ strains appear to utilize oleic acid as well as wild type [102]. Comparison of PHA monomer composition produced in *dci1*Δ and wild type cells grown in media containing 10Z,13Z, nonadecadienoic acid revealed that only a minor fraction of fatty acids was degraded via a pathway implicating the $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase (H. Bogdawa and Y. Poirier, unpublished). Analysis of the degradation of the conjugated linoleic acid isomers 10E,12Z-octadecadienoic acid by the *S. cerevisiae* cells revealed the requirement of the 2,4-dienoyl-CoA reductase and the Δ^3,Δ^2-enoyl-CoA isomerase but not the $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase [103]. Furthermore, studies using PHA to analyze the degradation of 9Z,11E-octadecadienoic acid revealed that efficient catabolism of such a conjugated fatty acid was largely independent of the $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase but instead relied on the presence of a Δ^3,Δ^2-enoyl-CoA isomerase [104]. This study indicated that while the conversion of the intermediate 3Z,5E-dienoyl-CoA to 2E,5E-dienoyl-CoA by the Δ^3,Δ^2-enoyl-CoA isomerase may be less thermodynamically favorable compared to its conversion to 2E,4E-dienoyl-CoA by the $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase, the main flux in yeast occurs through the former pathway [105,106].

A plant gene encoding a peroxisomal $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase in *A. thaliana* has recently been described [107]. The AtDCI1 protein exhibited approximately 38% and 18% amino acid identity to the mammalian and yeast $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase, respectively. The plant protein was shown to have $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase activity *in vitro* and to be able to complement *in vivo* the yeast *dci1*Δ mutant in a PHA assay using 10Z,13Z-nonadecadienoic acid.

6.5. α-Methylacyl-CoA racemase (Amacr)

The α-oxidation of β-methyl-branched fatty acyl-CoAs, which takes place in peroxisomes yields both R- and S-isoforms of α-methyl-branched fatty acids and furthermore, all bile acid intermediates are in the R-configuration. However, the first enzymes in the β-oxidation cycle are stereospecific for S-isoforms of their substrates [108]. Therefore, R-isoforms have to be converted to the S-orientation by Amacr, first described by Schmitz and Conzelmann [109] before β-oxidation. The substrate specificity of Amacr is rather broad. Generally speaking enzymes are known to have high enantiomeric specificity, and thus, Amacrs are an exception to this, as they interconvert *R* and S forms of a chiral centre.

Recently, the crystal structure of Amacr homologue from *Mycobacterium tuberculosis*, referred to as MCR, has been determined at the 1.8Å resolution [110]. The structure of this enzyme has confirmed that it belongs to the superfamily III CoA transferases. MCR is a dimeric enzyme, where the two subunits are tightly interlocked, because the C-terminal extension folds back on the N-terminal domain, such as to generate a large hole which is filled by protruding helices of the other subunit [110].

Mammals have one gene encoding an Amacr protein which has both N-terminal mitochondrial and C-terminal peroxisomal

targeting sequences [111] resulting in both mitochondrial and peroxisomal locations of Amacr in mammals. Patients with Amacr deficiency have been described [112]. Amacr has also recently been identified as a tissue biomarker for prostate [113] and colon cancer tissues [114].

An *Amacr*$^{-/-}$ mouse strain has been generated and partially analyzed [57]. As the mouse strain is not able to carry out Amacr-dependent chain shortening of the side chain of cholesterol during bile acid synthesis, this knock-out strain was assumed to represent "life without bile acids". The bile acid levels in the mouse strain were reduced to about 20% of that in *Amacr*$^{+/+}$, while the C27 precursors reached a level ~100 times higher than the controls. The *Amacr*$^{-/-}$ mice were surprisingly symptomless clinically when kept on standard laboratory diet. However, when the diet of *Amacr*$^{-/-}$ mice was fortified with phytol, some *Amacr*$^{-/-}$ mice died within 2 weeks. This proposed that the key function of Amacr is the elimination and detoxification of methyl-branched fatty acids. The remaining bile acids demonstrate the metabolic redundancy in bile acid synthesis, allowing essential lipid absorption.

7. Comments and future aspects

Our understanding of the β-oxidation cycle has increased significantly in the last years, particularly through the use of genetics and structural biology. However, there are still numerous gaps that need to be filled in. Future research should focus on getting a deeper understanding of the physiological contribution of the various enzymes participating in β-oxidation of fatty acids. For example, the physiological contribution of the evolutionary conserved $\Delta^{3,5}$,$\Delta^{2,4}$-dienoyl-CoA isomerase remains enigmatic in all organisms, even in the relative simple model system of *S. cerevisiae*. Concerted efforts on using several model systems to study the physiological contribution of β-oxidation should be maintained and expanded, as such contributions may be distinct between organisms. Furthermore, heterologous expression of β-oxidation enzymes in model systems, such as yeast, is a powerful tool that can be used to uncover functions. Deciphering the structure–function relationships of the β-oxidation enzymes is also likely to make key contributions leading to our understanding of their role in metabolism. MFEs are of special interest in this context. In mammals, a better understanding of the physiological function of β-oxidation and of the patho-physiology associated with mutations in the relevant genes is essential if the long-term goal of therapy is to be met. In plants, it will be interesting to know how β-oxidation contributes to seed germination as well as to the development of the embryo and flower. Such research may lead to the identification of novel signaling molecules that are generated or degraded via the β-oxidation cycle.

Acknowledgements

The original work at our laboratories were supported by grants from the Academy of Finland to JKH and TG, Sigrid Juselius Foundation, and by the F6P European Union Project "Peroxisomes" (LSHG-CT-2004-512018) to JKH, and the Fonds National Suisse (grant 3100A0-105874), the Herbette Foundation and the University of Lausanne to YP.

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